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# Effects of Dietary ω3 and ω6 Fatty Acids on the Fatty Acid Composition of RBC and Brain Synaptosomal, Microsomal and Mitochondrial Phospholipids and on Behavioral Development of Rats

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## ABSTRACT

The supply of different fatty acids during the developmental period has significant effects. This study examined the effects of dietary ω3 and ω6 fatty acid compositions on phospholipids (PLs) of RBC and rat brain subcellular fractions (synaptosome, microsome, mitochondria), and on learning ability of the 2nd generation rat. Rats were fed experimental diets 3 – 4 wks prior to the conception. Early in the lactation period, the feeding mothers were exchanged. Diets consisted of 10% fat (by weight), which was either safflower oil ('S') poor in ω3 fatty acids or computer-searched mixed oil ('M') with P/M/S ratio, 1/1.4/1 and ω6/ω3 ratio, 6.1/1. The 'S' and 'M' rats were subdivided further into SS, SM, MS & MM rats according to their lactation status. At 3 (weaning) & 9 wks of age, the percentage of total ω3 fatty acids and the ratios of ω3/ω6 fatty acids in PLs of RBC and brain subcellular fractions in SM and MM groups fed milk from the mixed oil-fed mothers for 2 wks tended to be higher than those in SS and MS groups respectively. In contrast, the concentrations of ω6 fatty acids, especially 22 : 5ω6 in all fractions, were significantly lower in the SM & MM groups compared to those of the SS & MS groups. The values for the DHAω3/22 : 5ω6 ratios after the lactation period were markedly higher in the groups (SM & MM) which were reared by mixed oil (MO) fed mothers. In carring out Y-water maze at 9th wk of age, the SM ($4.2 \pm 0.5$) & MM ($5.3 \pm 0.5$) groups made significantly less errors compared to the SS ($6.2 \pm 0.6$, $p < 0.05$ compared with SM) & MS ($7.2 \pm 0.5$, $p < 0.05$ compared with MM) groups which were lactated by the safflower oil-fed mothers. Therefore, by feeding a balanced fatty acid diet from the lactation period up to 9 wks of age, it was possible to differenciate visually- discriminating ability at 9 wks of age as compared with the groups fed ω3 fatty acid- deficient diet regardless of mother's diet given before parturition. The levels of DHA (synaptosome) and 22 : 5ω3 (mitochondria) were positively correlated not only with these values in RBC but also with visual discriminating ability. The levels of DHA and 22 : 5ω3 in RBC can, therfore, reflect visual discriminatng ability in the rat. (*Korean J Nutrition* 29(8) : 849~860, 1996)

**KEY WORDS** : docosahexaenoic acid (DHA) & arachidonic acid (AA) · lactation · brain subcellular fractions (synaptosome, microsome, mitochondria) · red blood cell (RBC) · behavioral development.

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## Introduction

The fact that docosahexaenoic acid(DHA, ω3 series) and arachidonic acid(AA, ω6 series) accumulate rapidly in phospholipids of cell membrane of the brain during development[1)2)] suggests that the availability of these fatty acids during this time may be crucial. There exists differences between species in terms of the time of the maximum growth of the brain ; it occurs in human during the last trimester of the gestation period and subsequent 18 months after birth[3)], in contrast to the occurance during postnatal lactation period in rats[4)]. The status of lactating rat is, therefore, similar to the preterm infant. Limited accretion of DHA to brain lipids is known to be related to alterations in visual response and learning behavior in rats[5-9)].

Babies born prematurely are most likely to have inadequate levels of DHA and AA in their brain. Thus, disorders of brain development can be permanent due to the fact that the brain cell neurons can not be regenerated once their cell proliferation is completed[10-12)]. Proper provision of essential nutrients such as DHA and AA become very important during this critical period.

This study examined the effects of diets either with desirable ratios of ω6/ω3 and P/M/S(mixed oil-fed group) or with deficient in ω3 series fatty acids(safflower oil-fed group) on the fatty acid compositions in RBC and brain synaptosomal, mitochondrial & microsomal phospholipids, and on behavioral development of the rat. The desirable fatty acid composition was computer-searched with different fats and oils to meet right ratios of both ω6/ω3 and P/M/S. Diets were fed 3-4 weeks before conception and new-born pups were fed maternal milk from the same or different mothers until 3 weeks of age.

## Materials and Methods

### 1. Animals and diets

Female Sprague-Dawley rats weighing 170-230g were divided into two groups and fed the experimental diets 3-4 weeks prior to their conception. Maternal experimental diets consisted of 10% fat(by weight), which was either safflower oil('S') poor in ω3 fatty acid or mixed oil('M') with P/M/S ratio, 1/1.4/1 and ω6/ω3 ratio, 6.1/1. This mixed oil with the desirable ratio of P/M/S and ω6/ω3 was chosen from various combinations of oils generated by a self-developed computer program. The mixed oil consisted of menhaden oil, soybean oil, corn oil, canola oil, palm oil which are 5%, 5%, 20%, 25%, and 45% respectively(weight%). The purified menhaden oil containing 1g of tocopherol acetate per kg oil was donated by the Zaphata Haynie Corp.(U.S.A.). The purified corn oil & canola oil without added extra antioxidants were donated by the Sam Yang Co., Ltd. (Seoul, Korea) ; the purified soybean oil without added extra antioxidants by the Dong Bang Co., Ltd. (Seoul, Korea) ; the purified palm oil without added extra antioxidants by the Nhong Shim Co., Ltd. (Seoul, Korea). The composition of maternal experimental diets and their fatty acid contents are shown in Table 1.

Three days after delivery, the litters were adjusted

**Table 1.** Composition(wt %) of experimental diets

| Ingredients | Mixed oil('M') | Safflower oil('S') |
|---|---|---|
| Carbohydrate[1)] | 65.0 | 65.0 |
| Mixed oill[2)] | 10.0 | – |
| Safflower oil | – | 10.0 |
| Others[3)] | 25.0 | 25.0 |
| 18:2ω6[4)] | 24.1 | 77.7 |
| 18:3ω3 | 2.4 | – |
| 20:4ω6 | 0.1 | 0.2 |
| 20:5ω3 | 0.5 | – |
| 22:6ω3 | 0.5 | – |
| Totalω3 | 3.9 | – |
| Totalω6 | 24.2 | 78.0 |
| Monounsaturates | 40.0 | 9.1 |
| Saturates | 28.0 | 11.4 |
| ω6/ω3 | 6.1 | – |
| P/M/S[5)] | 1.0/1.4/1.0 | 6.9/0.8/1.0 |

1) The carbohydrate was a mixture of 80% corn starch and 20% Sucrose.
2) Mixed oil (10% by wt) consisted of 0.5% menhaden oil, 0.5% soybean oil, 2.0% corn oil, 2.5% canola oil, and 4.5% palm oil (This mixture was selected from the computer-searched combinations of various fats and oils for this study).
3) Others contained 18% casein, 0.1% dl-methionine, 4% salt mixture & 1% vitamin mixture[13)] and 2% carboxymethyl cellulose.
4) The quantity of each fatty acid is given as a percent of the total fatty acids.
5) P/ M/ S: Polyunsaturated/ monounsaturated/ saturated fatty acids

to 8－10 animals. During the lactation period, 1 week after birth, the feeding mothers were exchanged to provide the pups for 2 weeks the milks of different fatty acid compositions from those of their natural mothers. Thus S and M rats were subdivided further into SS, SM, MS & MM groups according to their lactation status. After weaning(3 weeks of age), the young rats received the same diet as their mothers' until 9 weeks of age. Animals were sacrificed at 3 and 9 weeks of age to measure fatty acid composition of brain subcellular fractions at 3th & 9th week and to do Y-water maze test at 9th week.

Experimental design is given in Fig. 1.

### 2. Preparation of brain subcellular fractions

Twenty rats(10 samples) from each dietary group were sacrificed at 3th & 9th week. One sample consisted of two rats : one male & one female. Brain subcellular fractions were prepared by density gradient centrifugation applying a discontinuous sucrose gradient of three concentration steps, *ie.*, 0.32M, 0.8M and 1.2M : Brain synaptosomes were prepared according to Hajos[14], mitochondria according to Das & Ratty[15] and microsome according to Tahin et al.[16] The purity of the fractions was checked by electron microscopy[17] for synaptosome and mitochondria, and marker enzyme, *ie.*, cytochrome C oxidase[18] for mitochodria and microsome.

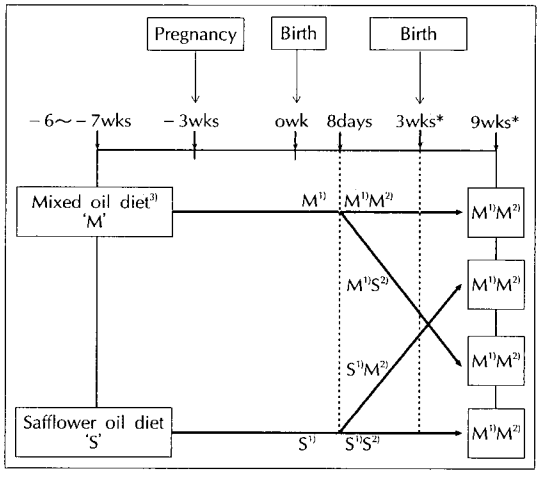


**Fig. 1.** The scheme of experimental design.
1) Diet status of mothers
2) Diet status of pups
3) Computer-searched mixture Oil with P/M/M=1/1.4/1 & ω6/ω3=6.1/1
* Sacrificed for measurements ; n=10(20 rats)/group

### 3. Lipid and fatty acid analysis

The lipids of brain subcellular fractions were extracted according to the method of Folch et al[19], and the lipids of RBC membrane according to the method of Way & Hanahan[20]. Total phospholipids (PLs) from brain subcellular fractions and RBC were separated by a thin layer chromatography(TLC). The silica gel areas were scraped off the plates immediately after the TLC procedure and methylated by the procedure of Lepage & Roy[21]. The compositions of fatty acid methyl esters were then measured by gas liquid chromatography(GLC, Hewlett-Packard 5890A). For the gas chromatographic separation, a bonded fused-silica capillary column(OMEGAWAX 320, Supelco, USA : 30m×0.32mm ID×0.25um) was used. The oven temperature of GLC was 200℃. The temperature of injection and detector ports was 260℃. Helium was used as the carrier gas for the column and the flow rate was 1ml/min with a split ratio of 30 : 1. Methyl esters of various fatty acids were identified by comparison with fatty acid methyl ester standards purchased from the Supelco(Catalog No. 1081) & Nu Chek Prep, Inc., USA(GLC-87A) and were then quantified on the basis of the amount of heptadecanoic acid internal standard purchased from Nu Chek Prep, Inc.(N-17-A).

### 4. Visual discrimination test in Y-water maze

A visual discrimination test in the Y-water maze at 9 weeks of age was carried out. The procedure was derived from that of Lamptey & Walker[5] and slightly modified by our laboratory[22]. The Y-water maze consisted of an escaped platform and two gates, one white and one black. The animals were trained in the maze for four days prior to the test period. On the first and second days, the rat was permitted to enter the white gate, where the escaped platform was located(positive response), or to enter the black gate, where no escaped platform was placed(negative response). The animal was permitted six positive and four negative runs in each of the 10-trial training sessions on the first two days. On the third and fourth training days, four trial runs were conducted and the animals were corrected for their wrong responses. Dur-

ing the following six consecutive days, each rat was permitted six trials a day and the negative trials were recorded as incorrect responses, *ie.*, errors. The mean of the cummulative number of errors over the 6 day test period were employed in comparing performances among different experimental groups. For each group 30 rats were tested in the Y-water maze.

### 5. Statistics

Statistical analysis was done using SAS procedure ; the results of fatty acid analyses were presented as mean±SEM and analyzed by one-way analysis of variance(ANOVA) ; the results of Duncan's Multiple Test were presented ; and Pearson's Correlation Analysis was performed and results were presented with p-values.

## Results

### 1. Brain growth

DNA concentrations of the brain rapidly increased and reached its maximum values at the 3rd week after birth and was leveled off since then(Fig. 2). Although DNA level in rat pup brains at 1 week of age lower in groups born from the SO-fed mothers than those born from the MO-fed mothers, the difference between mean values was not significant. At 9 weeks of age, the differences in the brain DNA concentration were not seen except for the SS group which was significantly lower than the other experimental group($p<0.05$).

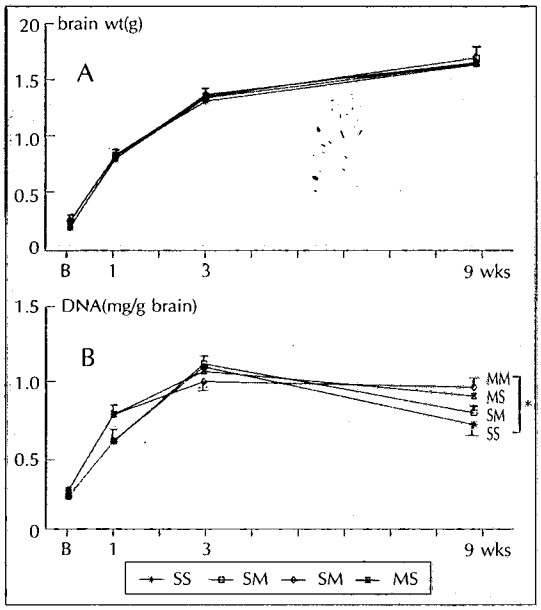


**Fig. 2.** Changes in brain weight(A) and concentration of DNA(B) in the rat pups during experimental periods. Results are the means±SEM from 30 animals(*$p<0.05$).

### 2. Fatty acid composition of rat milk

The fatty acid compositions of the day-8 milk from mixed oil (MO)-fed and safflower oil(SO)-fed rats are shown in Table 2. The percentage of DHA in MO-fed mothers milk was 0.55% and the average ratio of ω6/ω3 fatty acids was 7.14. The comparative values for DHA and ω6/ω3 fatty acid ratio of the MO-diet were 0.5% and 6.1 respectively. The mothers fed safflower oil(SO) based diet from 3 weeks before the conception produced milk containing 0.13% DHA, even though safflower oil contains no DHA.

### 3. Phospholipid-fatty acids of brain and RBC

As shown in Table 3, at 9 weeks of age, the composition of DHA and docosapentaenoic acid(22 : 5ω6) in phospholipids of brain subcellular fractions and RBC in groups MM & SM, which were fed MO-mother's milk until 3 weeks old and MO diet up to 9 weeks of age, were significantly different from those of groups SS & MS, which were fed SO-mother's

**Table 2.** Fatty acid compositions (%) of the rat milk at lactation day-8

| Fatty acids[1] | 'M'[2] | 'S'[3] |
|---|---|---|
| Saturates | 43.8 ±2.64 | 44.0 ±1.65 |
| Monounsaturates | 35.7 ±1.77 | 14.9 ±0.42* |
| Polyunsaturates | 18.3 ±0.80 | 38.0 ±1.33* |
| 18 : 2ω6 | 13.8 ±0.62 | 32.9 ±0.94* |
| 20 : 3ω6 | 0.40±0.03 | 0.89±0.06* |
| 20 : 4ω6 | 1.14±0.09 | 2.05±0.28* |
| 22 : 4ω6 | 0.23±0.06 | 0.69±0.18* |
| 22 : 5ω6 | 0.32±0.06 | 0.40±0.04 |
| Totalω6 | 16.1 ±0.71 | 37.5 ±1.30* |
| 18 : 3ω3 | 0.85±0.03 | 0.33±0.01* |
| 20 : 5ω3 | 0.47±0.04 | 0.05±0.01* |
| 22 : 5ω3 | 0.34±0.05 | 0.08±0.02* |
| 22 : 6ω3 | 0.55±0.05 | 0.13±0.04* |
| Totalω3 | 2.20±0.09 | 0.56±0.06* |
| ω6/ω3 | 7.14 | 50.0 |
| P/M/S[4] | 0.44/0.85/1.0 | 0.88/0.34/1.0 |

1) Results are means ± SEM expressed as wt % of total fatty acids from seven independent analyses(*$p<0.05$).
2) 'M' : Milk of mother group fed mixed oil diet
3) 'S' : Milk of mother group fed with ω3 fatty acid deficient diet
4) P/M/S : Polyunsaturated/monounsaturated/saturated.

**Table 3.** Compositions(%) of the major ω6 and ω3 fatty acids phospholipids of RBC and brain subcellular fractions[1]

| | 18 : 2ω6 | | 20 : 4ω6 | | 22 : 4ω6 | | 22 : 5ω6 | | 20 : 5ω3 | | 22 : 4ω3 | | 22 : 6ω3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 wsk | 9 wks | 3 wsk | 9 wks | 3 wsk | 9 wks | 3 wsk | 9 wks | 3 wsk | 9 wks | 3 wsk | 9 wks | 3 wsk | 9 wks |
| RBC phospholipids | | | | | | | | | | | | | | |
| SS | 9.3±0.22$^{bc}$ | 9.9±0.09$^{b}$ | 17.9±0.71$^{b}$ | 19.8±0.39$^{b}$ | 2.57±0.14$^{a}$ | 2.14±0.09$^{b}$ | 1.66±0.08$^{a}$ | 1.35±0.06$^{a}$ | 0.31±0.07$^{b}$ | 0.06±0.00$^{c}$ | 0.60±0.11$^{d}$ | 0.62±0.02$^{b}$ | 2.00±0.15$^{d}$ | 1.21±0.03$^{b}$ |
| MS | 11.5±0.27$^{a}$ | 10.7±0.36$^{a}$ | 19.2±0.27$^{a}$ | 21.9±0.38$^{a}$ | 2.56±0.09$^{a}$ | 2.78±0.17$^{a}$ | 0.87±0.10$^{b}$ | 1.46±0.13$^{a}$ | 0.07±0.01$^{c}$ | 0.07±0.03$^{c}$ | 1.73±0.14$^{c}$ | 0.75±0.08$^{b}$ | 3.60±0.10$^{c}$ | 1.51±0.22$^{b}$ |
| SM | 9.9±0.16$^{b}$ | 9.1±0.20$^{c}$ | 16.9±0.27$^{b}$ | 19.9±0.26$^{b}$ | 1.46±0.04$^{b}$ | 1.46±0.73$^{c}$ | 0.39±0.06$^{c}$ | 0.53±0.10$^{b}$ | 0.71±0.04$^{a}$ | 0.67±0.09$^{b}$ | 2.53±0.10$^{b}$ | 2.16±0.14$^{a}$ | 5.09±0.11$^{b}$ | 3.41±0.16$^{a}$ |
| MM | 8.8±0.30$^{c}$ | 8.7±0.11$^{c}$ | 17.6±0.40$^{b}$ | 18.1±0.51$^{c}$ | 1.15±0.06$^{c}$ | 0.82±0.02$^{b}$ | 0.26±0.02$^{c}$ | 0.24±0.02$^{c}$ | 0.68±0.04$^{a}$ | 1.04±0.06$^{a}$ | 2.87±0.09$^{a}$ | 2.22±0.11$^{a}$ | 6.18±0.13$^{a}$ | 3.52±0.10$^{a}$ |
| Synaptosomal phospholipids | | | | | | | | | | | | | | |
| SS | 1.39±0.04 | 0.94±0.04$^{b}$ | 11.6±0.26$^{c}$ | 9.39±0.19 | 2.85±0.15$^{c}$ | 3.54±0.10$^{a}$ | 3.35±0.10$^{a}$ | 3.35±0.19$^{a}$ | 2.55±0.07$^{a}$ | 0.06±0.00 | 0.23±0.01 | 0.17±0.02 | 8.2±0.36$^{d}$ | 12.8 ±0.35$^{d}$ |
| MS | 1.51±0.04 | 1.05±0.04$^{a}$ | 13.3±0.25$^{a}$ | 9.39±0.33 | 3.58±0.11$^{a}$ | 3.33±0.15$^{ab}$ | 1.61±0.13$^{b}$ | 1.99±0.03$^{b}$ | 0.60±0.00 | 0.25±0.02 | 0.16±0.05$^{ab}$ | 0.20±0.05 | 13.1±0.40$^{c}$ | 13.4 ±0.49$^{c}$ |
| SM | 1.39±0.04 | 0.82±0.03$^{c}$ | 12.5±0.28$^{b}$ | 9.63±0.13 | 3.06±0.12$^{bc}$ | 3.14±0.17$^{b}$ | 1.27±0.11$^{b}$ | 0.78±0.08$^{c}$ | 0.04±0.03 | 0.25±0.01 | 0.16±0.03$^{ab}$ | 0.24±0.02 | 14.5±0.72$^{b}$ | 15.53±0.53$^{b}$ |
| MM | 1.38±0.08 | 0.79±0.02$^{c}$ | 12.9±0.22$^{ab}$ | 9.39±0.12 | 3.19±0.05$^{b}$ | 3.07±0.09$^{b}$ | 0.53±0.06$^{c}$ | 0.44±0.04$^{c}$ | 0.05±0.02 | 0.23±0.02 | 0.22±0.03$^{a}$ | 0.27±0.03 | 15.9±0.41$^{a}$ | 16.5±0.26$^{a}$ |
| Microsomal phospholipids | | | | | | | | | | | | | | |
| SS | 0.67±0.04$^{b}$ | 0.57±0.02$^{a}$ | 9.6±0.14$^{b}$ | 3.16±0.10$^{b}$ | 3.22±0.10$^{a}$ | 3.47±0.12$^{a}$ | 2.49±0.09$^{a}$ | 0.04±0.01$^{c}$ | 0.19±0.01$^{b}$ | 0.16±0.02$^{ab}$ | 0.15±0.02$^{b}$ | 0.15±0.02$^{b}$ | 8.54±0.45$^{c}$ | 13.2 ±0.43$^{d}$ |
| MS | 0.76±0.02$^{a}$ | 0.61±0.04$^{a}$ | 10.5±0.20$^{a}$ | 9.20±0.17$^{a}$ | 3.57±0.19$^{a}$ | 3.26±0.14$^{a}$ | 1.64±0.12$^{b}$ | 2.34±0.26$^{a}$ | 0.03±0.01 | 0.17±0.03$^{c}$ | 0.15±0.01$^{b}$ | 0.17±0.01$^{b}$ | 12.8±0.68$^{b}$ | 14.7±0.29$^{c}$ |
| SM | 0.62±0.02$^{bc}$ | 0.44±0.01$^{b}$ | 10.1±0.10$^{a}$ | 8.78±0.12$^{b}$ | 3.23±0.07$^{b}$ | 2.97±0.06$^{ab}$ | 1.40±0.08$^{b}$ | 0.75±0.12$^{b}$ | 0.04±0.01 | 0.24±0.02$^{a}$ | 0.20±0.01$^{a}$ | 0.28±0.02$^{a}$ | 14.3±0.43$^{a}$ | 17.5±0.41$^{b}$ |
| MM | 0.57±0.01$^{c}$ | 0.42±0.02$^{b}$ | 10.4±0.10$^{a}$ | 8.53±0.07$^{b}$ | 3.35±0.07$^{ab}$ | 2.80±0.10$^{b}$ | 0.76±0.02$^{c}$ | 0.41±0.03$^{b}$ | 0.04±0.02 | 0.22±0.01$^{a}$ | 0.19±0.02$^{ab}$ | 0.28±0.01$^{a}$ | 15.7±0.31$^{a}$ | 18.5±0.15$^{a}$ |
| Mitochodrial phospholipids | | | | | | | | | | | | | | |
| SS | 1.92±0.06 | 1.71±0.06$^{a}$ | 13.3±0.68 | 12.4±0.16$^{a}$ | 2.64±0.14 | 3.09±0.8$^{a}$ | 3.02±0.16$^{a}$ | 2.69±0.08$^{a}$ | 0.05±0.01 | 0.23±0.01 | 0.09±0.01$^{b}$ | 0.15±0.01$^{b}$ | 8.4±0.53$^{c}$ | 16.0±0.43$^{c}$ |
| MS | 2.02±0.11 | 1.61±0.06$^{a}$ | 14.1±0.25 | 12.4±0.18$^{a}$ | 2.98±0.11 | 3.02±0.08$^{ab}$ | 1.45±0.08$^{b}$ | 2.24±0.24$^{b}$ | 0.04±0.00 | 0.27±0.05 | 0.08±0.02$^{b}$ | 0.02±0.02$^{b}$ | 12.5±0.40$^{b}$ | 16.7±0.49$^{c}$ |
| SM | 1.89±0.03 | 13.5±0.04$^{b}$ | 14.0±0.18 | 11.8±0.12$^{b}$ | 2.92±0.20 | 2.84±0.07$^{b}$ | 1.25±0.13$^{b}$ | 0.79±0.11$^{c}$ | 0.07±0.01 | 0.02±0.26 | 0.20±0.02$^{a}$ | 0.33±0.03$^{a}$ | 15.5±0.33$^{a}$ | 18.7±0.40$^{b}$ |
| MM | 1.87±0.08 | 1.45±0.05$^{b}$ | 14.2±0.20 | 12.2±0.20$^{ab}$ | 2.86±0.08 | 2.41±0.05$^{c}$ | 0.56±0.04$^{c}$ | 0.37±0.04$^{d}$ | 0.06±0.01 | 0.29±0.02 | 0.22±0.03$^{a}$ | 0.30±0.01$^{a}$ | 16.5±0.47 | 19.9±0.38$^{a}$ |

1) Results are means±SEM expressed as wt % of total fatty acids from ten independent analyses(20 rats) per each group.
Superscripts a, b, c indicate that values with the different letters are significantly different from the same fatty acid and same fraction : $p<0.05$.

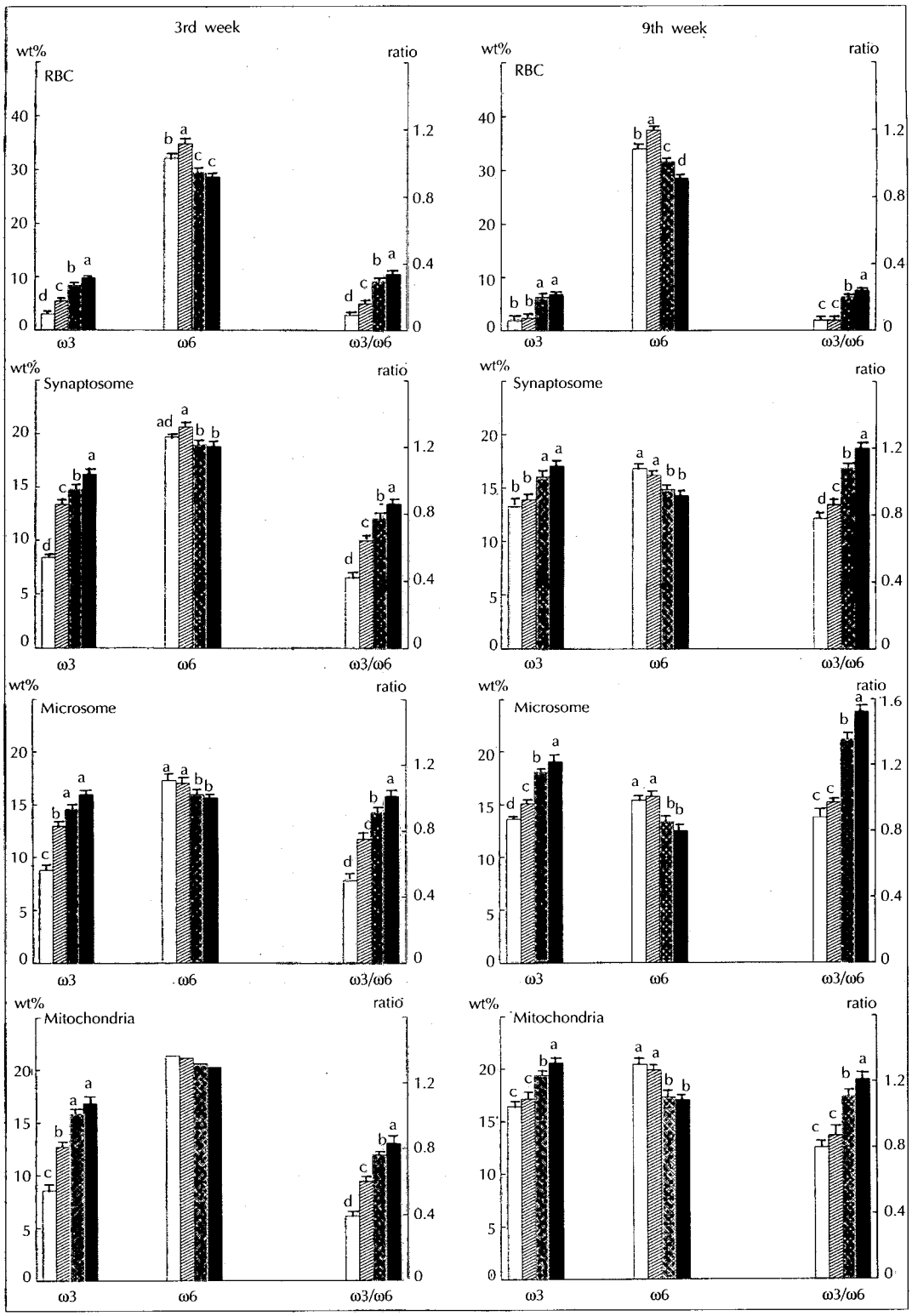


**Fig. 3.** The total ω3 & ω6 long chain polyunsaturated fatty acids(LCPs), and the ratio of ω3/ω6 LCPs in PLs of RBC and brain subcellular fractions in rat at the age of 3 & 9 weeks. □ SS ; ▨ MS ; ▩ SM ; ■ MM. Mean±SEM ; n=10(20 rats)/group. Letters a, b and c indicate that the values with the different letters are significantly different from the others : $p<0.05$.

milk until 3 weeks old and SO diet up to 9 weeks of age. The relative amount of DHA in rat pups brain was increased by changing from SS to SM and was counterbalanced by a decrease in 22 : 5ω6. This counterbalancing phenomenon between DHA and 22 : 5 ω6 was also observed in RBC. On the other hand, the differences in the composition of AA in phospholipids of the rat brain in all experimental groups are relatively small as compared to those in other LCFA's at 9 weeks of age, *ie.*, DHA and 22 : 5ω6 mentioned above.

The compositions of ω3 & ω6 fatty acids in phospholipids of brain subcellular fractions and RBC are shown in Fig. 3. The percentages of total ω3 fatty acids, especially including DHA in brain synaptosome, microsome and mitochondria were in the increasing order of SS<MS<SM<MM and the same order was also seen in RBC phospholipid-fatty acids. Since the opposite trend was seen in total ω6 fatty acid compositions, the order of ω3/ω6 fatty acid ratios appeared as SS<MS<SM<MM both in brain subcellular fractions and RBC.

For those groups which were reared by mothers fed

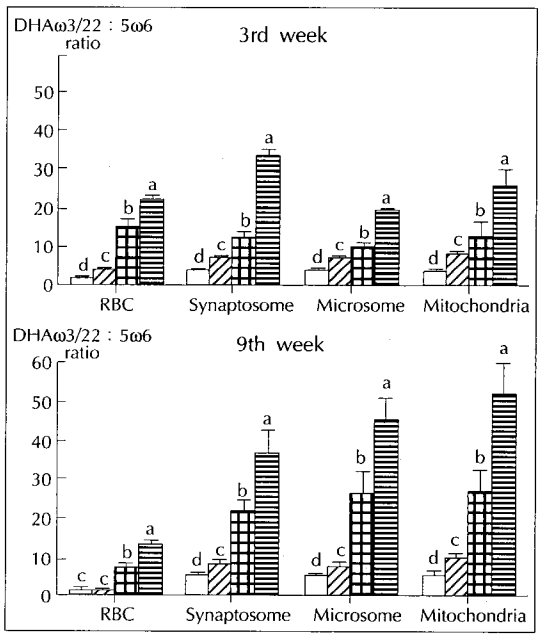


**Fig. 4.** The ratio of DHA/22 : 5ω6 in PLs of RBC and brain subcellular fractions at the age of 3 & 9 weeks in rat. □ SS ; ▨ MS ; ■ SM ; ▤ MM. Mean±SEM ; n= 10(20 rats)/group. Letters a, b and c indicate that the value with the different letters are significantly different from the others : $p<0.05$.

MO diet, *ie.*, MM and SM rats, 22 : 6 ω3/22 : 5ω6 ratios at 9 weeks of age were significantly higher than those for SS and MS groups reared by SO-fed mothers(Fig. 4). These ratios in brain subcellular fractions tended to be higher at 9 weeks of age than at 3 weeks of age, in contrast to the opposite trend in RBC.

As shown in Table 4, ω3 and ω6 fatty acid compositions in phospholipids of RBC were positively correlated with those in brain synaptosomal, microsomal and mitochondrial fractions. Among ω3 series fatty acids, DHA was highly correlated($r=0.706-0.805$) and 22 : 5ω6 showed a highest correlation($r=0.861-0.875$) in ω6 series fatty acids.

### 4. Y-water maze

In the visual discrimination test carried out in Y-water maze at 9weeks of age, the SM(4.16±0.46) and MM(5.29±0.53) groups reared by MO-fed mothers since 8 days after birth, made significantly less errors compared to groups SS(6.24±0.64) and MS(7.19±0.53) reared by SO-fed mothers instead of MO-fed mothers($p<0.05$ between SM & SS, MM & MS, MS & SM, Fig. 5).

### 5. Correlation between phospholipd-fatty acids and behavior

According to the Pearson's Correlations Test between phospholipid-fatty acid compositions and Y-water maze results, *ie.*, the number of errors made(Table 5), the percentage of DHA(synaptosome), 22 : 5ω3 (mitochondria) and 18 : 1ω9(microsome) in phos-

**Table 4.** Correlation matrix between fatty acid compositions of RBC and brain subcellular fractional phospholipids in 9 weeks old rats[1)]

| Fatty Acids | Synaptosome | Microsome | Mitochondria |
|---|---|---|---|
| 18 : 3ω3 | 0.605* | 0.323 | −0.074 |
| 20 : 53 | 0.088 | 0.404 | 0.358 |
| 22 : 5ω3 | 0.444* | 0.779* | 0.818* |
| 22 : 6ω3 | 0.792* | 0.706* | 0.805* |
| Total ω3 | 0.783* | 0.721* | 0.823* |
| 18 : 2ω6 | 0.549* | 0.500* | 0.641* |
| 20 : 4ω6 | −0.062 | −0.059 | 0.346 |
| 22 : 4ω6 | 0.380* | 0.603* | 0.531* |
| 22 : 5ω6 | 0.875* | 0.874* | 0.861* |
| Total ω6 | 0.463* | 0.641* | 0.723* |
| ω3/ω6 | 0.868* | 0.878* | 0.899* |

1) Correlation coefficients(r) are presented with p-value ; *$p<0.05$.

pholipids of the brain subcellular fractions were negatively correlated, and 22 : 5ω6 and 18 : 2ω6 were positively correlated to the number of errors made, indicating the long chain ω3 series fatty acids affected favorably the behavioral development of the rat. Interestingly, in brain synaptosome, the precusors of long chain ω6 and ω3 fatty acids, *ie.*, 18 : 2ω6(r=−0.4408) and 18 : 3ω3(r=−0.4666) significantly increased, and the product, DHA(r=−0.3028) decreased the number of errors made in Y-water maze. Among monounsaturated fatty acids, while 18 : 1 (microsome, r=−0.4573) significantly decreased, 24 : 1(microsome, r=0.3108), 22 : 1(mitochondria, r=0.5123) and 16 : 1(synaptosome, r=0.3288) significantly increased the number of errors made.

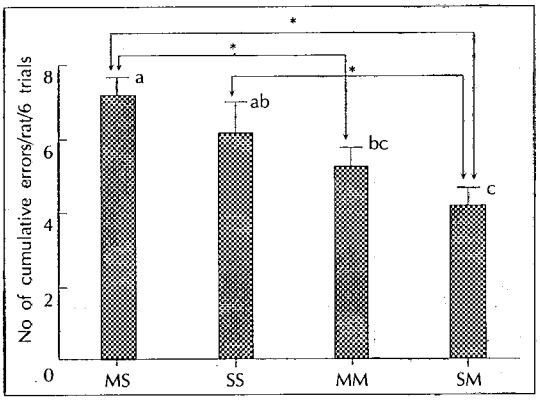


**Fig. 5.** Cumulative errors in the visual discrimination test(Y-water maze) at the age of 9 weeks in rat. Mean± SEM ; n=30 rats/group. Letters a, b and c indicate that the value with the different letters are significantly different from the others : *p<0.05.

**Table 5.** Correlation matrix between fatty acid compositions of the brain subcellular fractional phospholipids and Y-water maze test in 9 weeks old

| Fatty Acids | Synaptosome | Microsome | Mitochondria |
|---|---|---|---|
| 14 : 0 | 0.4072** | −0.3101 | 0.1629 |
| 16 : 0 | −0.0479 | −0.2270 | −0.0369 |
| 16 : 1 | 0.3288* | −0.1465 | −0.0411 |
| 18 : 0 | −0.2190 | −0.2035 | 0.0319 |
| 18 : 1 | −0.0821 | −0.4573** | −0.0843 |
| 18 : 2ω6 | 0.4408** | 0.2607 | 0.2284 |
| 18 : 3ω3 | 0.4066* | −0.0763 | 0.2197 |
| 20 : 0 | −0.0896 | 0.0484 | 0.0657 |
| 20 : 1 | −0.1986 | 0.2690 | 0.2097 |
| 20 : 3 | −0.2898 | 0.0300 | 0.0973 |
| 20 : 4ω6 | −0.1892 | 0.1122 | 0.2088 |
| 20 : 5ω3 | −0.0366 | −0.2080 | −0.1919 |
| 22 : 0 | 0.0150 | 0.2045 | −0.1041 |
| 22 : 1 | −0.0784 | 0.1230 | 0.5123* |
| 22 : 4ω6 | 0.0233 | 0.1512 | −0.0028 |
| 22 : 5ω6 | 0.1553 | 0.2608 | 0.2448 |
| 22 : 5ω3 | −0.1380 | −0.3041 | −0.4084* |
| 24 : 0 | −0.0951 | 0.1442 | −0.3093 |
| 22 : 6ω3 | −0.3028* | −0.2326 | −0.2190 |
| 24 : 1 | 0.0399 | 0.3108* | −0.1300 |
| DHA/AA | −0.2336 | −0.2479 | −0.2780 |
| DHA+EPA/AA | −0.2130 | −0.3000 | −0.2701 |
| Longer ω3[2] | −0.1839 | −0.2808 | −0.1648 |
| Longer ω6[3] | 0.0236 | 0.2306 | 0.2288 |

1) Correlation coefficients(r) are presented with p-alue ; *p< 0.05, **p<0.01.
2) Longer ω3 is sum of 20 : 5, 22 : 5 and 22 : 6
3) Longer ω6 is sum of 20 : 4, 22 : 4 and 22 : 5

## Discussion

The present study aimed to show the effects of dietary fats containing markedly different fatty acids on the compositions of fatty acids in brain subcellular fractions and RBC, and on the behavioral development of rat pups.

MO-mothers fed a computer-searched mixed oil diet with ω6/ω3 fatty acid ratio of 6.1 and DHA level of 0.5% from 3 weeks before the conception, produced milk with a DHA level of 0.55% and the ω6/ω3 ratio of 7.14. Although only a trace of EPA and DHA was found in the milk of SO-mothers fed ω3-deficient safflower oil diet, the brain phospholipids of these pups accumulated a considerable amount of DHA[23]. It seems that dietary ω3 fatty acid deficit during the gestation period can be offset partially by selective retention of DHA by dam, possibly from the liver [24]. Bazan et al[25] suggested that the liver is the organ which actively synthesizes DHA and send it to the brain. The maternal adipose tissue was also suggested as a reservoir and provider of DHA[26].

By changing mothers from day 8 of lactation either from S to M(SS→SM) or from M to S(MM→MS) it was possible to affect fatty acid compositions of phospholipids in brain synaptosome, microsome and mitochondria, and RBC of rat pups. The percentages of ω3 fatty acids in phospholipds tended to decrease in the order of MM>SM>MS>SS. The percentage of ω3 fatty acids and ω3/ω6 fatty acid ratios in total brain phospholipids showed a remarkable change by exchanging mothers during the lactation period when the growth of rat brain is at its maximum rate, *ie.*, the values of SM became significantly higher than those of MS. SM pups were born to the mothers fed the ω3

fatty acid deficient diet from 3 weeks before conception, *ie.*, exposed to SO-diet for 7 weeks and then fed MO-mothers milk for only 2weeks from the first week after birth(Fig. 3). The ω3/ω6 fatty acid ratios of rat pups' synaptosome, microsome and mitochondria at 9 weeks of age were 1.1 – 1.2, 1.4 – 1.5, and 1.1 – 1.2 respectively. These ratios of RBC at 9 weeks of age were 0.2 – 0.3. One interesting observation was that ω3/ω6 fatty acid ratios increased from 3weeks to 9 weeks after birth in the brain subcellular fractions, while the values decreased in RBC. This phenomenon seemed to indicate that the supply of ω3 fatty acids in the brain had already decreased at 9 weeks of age.

As suggested from the previous works[6)27-34)], the increase in DHA in rat pups brain caused by changing from SS to SM was counterbalanced by a decrease in 22 : 5ω6. This counterbalancing phenomenon between DHA and 22 : 5ω6 in rat brain was also seen in RBC. It seemed very important to observe that the SM- and MM-pups fed MO-mother's milk and MO-diet until 9 weeks of age showed significantly higher ratios of DHAω3/22 : 5ω6 than SS- or MS-pups fed SO-mother's milk and SO-diet until 9 weeks of age. The lower levels of DHA in brain subcellular fraction of groups fed milk of SO-mothers fed ω3 fatty acid deficient diet were accompanied by higher levels of 22 : 5ω6 and, therefore, lower DHAω3/22 : 5ω6 ratio. The ratio of DHAω3/22 : 5ω6 in brain subcellular fractions has been suggested to be the more sensitive indicator of the dietary ω3 fatty acid adequacy than percentage of DHA or 22 : 5ω6[27)] alone. Arbuckle et al.[32)] also suggested that the ratio of DHAω3/22 : 5ω6, especially in synaptic membranes was a more sensitive index of the adequacy of ω3 fatty acids in the formula than the percentage of DHA or 22 : 5ω6 alone, or the DHAω3/22 : 5ω6 ratio in brain total lipid.

Despite the change in brain DHAω3/22 : 5ω6 ratio, the total PUFA content in brain subcellular fractions appeared to become similar between experimental groups at 9 weeks of age. This apparent regulation of brain lipid unsaturation may be achieved by regulation of specific fatty acid uptake, desaturation, acylation or remodeling[35)].

The relative percentage of AA was also maintained at 9 weeks of age. The ability to maintain brain AA could be partly explained by the presence of specific, high affinity transport systems for AA into brain[36)]. Retroconversion of 22- to 20-carbon chain LCP has been suggested in the brain and might also be involved in maintaining a constant AA level[32)]. It is possible that 22 : 4ω6 serves as a pool of ω6 fatty acids to maintain optimal brain arachidonic acid levels[32)].

One other important observation made from this study is that the percentage of ω3 series fatty acid such as DHA and 22 : 5ω3 and ω3/ω6 ratio in RBC phospholipids were positively correlated with those values of brain subcellular fractions. DHA as a ω3 series fatty acid and 22 : 5ω6 as a ω6 series fatty acid in RBC were most highly correlated with those in brain subcellular fractions. A number of studies[10)31)35)] have suggested that alterations of DHA concentrations in RBC by dietary ω3 fatty acids may reflect parallel changes in other tissues, particularly the brain.

In this study, employing a visual discrimination test in Y-water maze, it was shown that by changing rat pups from SO-fed mothers to MO-fed mothers during lactation period when the rat brain grows fastest and by feeding the same diet until 9 weeks of age, it was possible to differentiate behavioral development, *ie.*, SM pups made less errors than SS or MS in Y-water maze at 9 weeks of age. The present observation is in agreement with Wainwright et al[37)] who found decreases in DHA and acquisition learning rate in morise maze by feeding mice ω3 deficient diets during pregnancy and lactation periods. The major brain growth spurt and lipid deposition occurs postnatal in the rat, but perinatal in human[3)4)38)]. The lactation period in the rat corresponds to the preterm period in humans. Special emphasis on nutrition support, therefore, should be made for the preterm babies.

In Pearson's Correlation Test(Table 5), ω3 series fatty acids such as DHA and 22 : 5ω3 in phospholipids of the brain subcellular fractions were negatively correlated and ω6 series fatty acids such as 22 : 5ω6 and 18 : 2ω6 were positively correlated with the number of errors made in Y-water maze test. In brain microsome, however, 18 : 1ω9 was negatively correlated and 24 : 1 was positively correlated with the number of errors made. The findings that the monounsaturates either increase or decrease the number of errors made and the higher percentage of the precursors

of ω3 and ω6 LCPUFA, *ie.*, 18 : 2ω6 and 18 : 3ω3 in synaptosome, the higher the number of errors made in visual discrimination test need clarification in further research.

In conclusion, by feeding a balanced fatty acid diet from the lactation period, it was possible to differentiate visually discriminating ability in behavioral development test at 9 weeks of age as compared with the groups fed the ω3 fatty acid deficient diet, regardless of the mother's previous diet given before parturition. The levels of DHA(synaptosome) and 22 : 5ω3(mitochondria) were positively correlated not only with these values in RBC but also with visual discriminating ability. The levels of DHA and 22 : 5ω3 in RBC can, therfore, reflect visual discriminatng ability in the rat.

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=국 문 초 록=

# 식이 ω3와 ω6계 지방산 조성이 제 2세대 쥐의 RBC과 뇌조직 Synaptosome, Microsome 및 Mitochondria의 인지질 및 행동발달에 미치는 영향

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뇌발달의 가장 활발한 시기가 사람의 경우에는 임신 3기~생후 18개월이며, 쥐의 경우 사람과 달리 수유기에 가장 활발하다. 따라서 이러한 주요한 시기에 ω3와 ω6계 지방산의 균형된 공급은 매우 중요하다.

본 연구에서는 지방산 조성을 달리한 식이를 어미 쥐에게 임신되기 3~4주전부터 섭취시키고, 이로 부터 출생한 새끼 쥐에게 출생 1 주부터 어미를 교체하여, 지방산 조성이 다른 모유를 수유기동안(2주간) 공급시키고 계속하여 9주까지 사육한 후, 제 2세대 쥐의 RBC와 뇌조직의 subcellular fraction인 synaptosome, microsome 및 mitochondria의 인지질 지방산 조성 및 행동발달에 미치는 영향을 조사하였다. 어미 식이의 지방은 10% 수준으로, 자체에서 개발한 전산화 프로그램으로 찾아 낸 바람직한 P/M/S(1 : 1.4 : 1)와 ω6/ω3(6.1) 비율을 가진 혼합유(mixed oil, 'M')군과 ω3계 지방산 결핍군인 safflower oil('S') 군으로 분류하였다. 제 2세대 쥐는 수유기동안 공급받은 모유의 종류에 따라 SS, SM, MS, MM 군으로 분류하였다. MM과 SM군의 경우, RBC와 뇌조직 subcellular fraction에서 총 ω3계 지방산과 ω3/ω6 비율은 생후 3주와 9주에 SS와 MS군보다 유의적으로 높게 나타난 반면, ω6계 지방산, 특히 22 : 5ω6 수준은 SS군과 MS군보다 유의적으로 낮았다. 수유후 DHAω3/22 : 5ω6 비율은 MM과 SM군에서 현저하게 증가되었다. 9주에 visual discrimination test를 실시한 결과, 임신기와 임신되기 3~4주전에 섭취한 어미의 식이와는 관계없이, 뇌발달 속도가 최대인 수유기동안 ω3와 ω6계 지방산을 바람직한 비율로 함유한 식이로 바꾸고 9주까지 계속하여 공급받은 경우, ω3계 지방산이 결핍된 식이를 섭취시킨 군과는 9주에 실시한 행동발달 test에서 유의적인 차이를 보여 주었다. RBC 인지질의 DHA 및 22 : 5ω3 수준은 뇌조직 subcellular fraction과 양의 상관관계를 나타내고 뇌조직 subcellular fraction 인지질의 DHA와 22 : 5ω3 수준은 행동발달(visual discriminating ability) 결과와 유의적인 관계를 나타내므로, RBC 인지질의 DHA 및 22 : 5ω3 수준이 본 연구에서 이용된 행동발달의 척도가 될 수 있음을 시사해 준다.